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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

2



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

November 13, 2003

MEMORANDUM

SUBJECT: **2,4-DB [4-(2,4-Dichlorophenoxy)butyric acid]**. Chemical 030801. HED Review of a Confined Rotational Crop Study with ¹⁴C-2,4-DB using Carrots, Lettuce and Barley (860.1850). DP Barcode D290502 [MRID 43004301].

FROM: Danette Drew, Chemist *DD*
Reregistration Branch 3
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THROUGH: Whang Phang, Branch Senior Scientist *WPhang*
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TO: Cecelia Watson, Chemical Review Manager
Special Reregistration and Review Division (7508C)

The 2,4-DB Task Force has submitted a confined rotational crop study for the determination and characterization of residues of 2,4-DB in rotated crops. This study has been evaluated in the attached Data Evaluation Record (filenames 43004301.der).

These data have been reviewed by Dynamac Corp. under contract to the Agency and have undergone secondary review in Reregistration Branch 3 of the Health Effects Division to reflect current policies.

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2,4-DB/PC Code 030801/The 2,4-DB Task Force

OPPTS 860.1850

Confined Accumulation in Rotational Crops - Barley, Carrot, and Lettuce

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Date: 9/29/03

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Date: 11/13/03

These data have been reviewed by Dynamac Corp.(8/21/03) under contract to the Agency and have undergone secondary review in Reregistration Branch 3 of the Health Effects Division to reflect current policies.

STUDY REPORT:

43004301 O'Neal, S. and Johnson, T. (1993) A Confined Rotational Crop Study with ^{14}C -2,4-DB Using Carrots (*Daucus carota*), Lettuce (*Lactuca sativa*), and Barley (*Hordeum vulgare*). Lab Project Number: 508. Lab Report Number: 1522. Unpublished study prepared by PTRL East, Inc. (Lexington, KY) and submitted by the 2,4-DB Task Force (Raleigh, NC). 310 p.

EXECUTIVE SUMMARY:

The 2,4-DB Task Force submitted the results of a confined rotational crop study with 2,4-DB. Uniformly ring labeled [^{14}C]2,4-DB was sprayed directly onto the soil surface of sandy loam soil at a target rate of 1.5 lb ai/A; the actual application rate was verified at 1.65 lb ai/A. Rotational crops consisting of barley (a small grain crop), carrot (a root crop), and lettuce (a leafy vegetable crop) were planted 30, 120, and 365 days after treatment (DAT). At appropriate crop growth stages, immature and mature samples of the rotated crops were collected from each plantback interval (PBI). The collected samples were stored frozen prior to residue characterization and identification. Sample storage conditions and intervals are supported by adequate storage stability data. The field phase of the study was conducted at a small fenced outdoor plot in Madera, CA, and the analytical phase was performed by PTRL East, Inc. (Richmond, KY).

The total radioactive residues (TRR; expressed as 2,4-DB equivalents) in/on treated rotational crop commodities were, overall, low and ranged from <0.01 ppm (365-DAT carrot root) to 0.07 ppm (120-DAT barley straw). There appears to be no discernible trend as to whether residues would increase or decrease at longer PBI because of low TRR.



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Radioactive residues in/on rotational crop commodities were sequentially extracted with a polar solvent (methanol:water) and a non-polar solvent (ethyl acetate) followed by hydrolysis with hydrochloric acid and sodium hydroxide. Extracts and hydrolysates which contained radioactivity level of >0.01 ppm were analyzed by adequate chromatographic techniques (HPLC and TLC).

The parent compound, 2,4-DB, was not detected in any plant matrix at any rotational interval. The metabolites identified in rotational crop samples include the phenols (2-chloro-4-hydroxyphenol; 2,4-dichlorophenol; 2,5-dichloro-4-hydroxyphenol; 2,4-dichloro-3-hydroxyphenol; and 2,3-dichloro-4-hydroxyphenol), the phenoxyacetic acids (2,3-dichloro-4-hydroxyphenoxyacetic acid; 2,5-dichloro-4-hydroxyphenoxyacetic acid; and 2,4-dichloro-3-hydroxyphenoxyacetic acid) and 1,4-benzoquinone. Each metabolite identified comprised <3% of TRR except for 2,4-dichlorophenol which was detected at 10.91% of TRR in 120-DAT barley forage. No single metabolite was observed at a residue level exceeding 0.01 ppm.

The remaining nonextractable residues of 365-DAT barley forage were subjected to cell wall fractionation in order to determine the nature of bound residues. The nonextractable residues were characterized to be associated with cellulose (~26% TRR) and hemicellulose (~7% TRR), with minor amounts of starch and pectin (each <1% TRR), protein (~2% TRR), and lignin (<2% TRR).

The confined rotational crop studies showed that no significant residues of 2,4-DB were taken up in succeeding rotational crops. The registrant proposed that the major metabolic pathways in rotational crop matrices encompassed side chain degradation, chlorine shift and ring hydroxylation as demonstrated by the observed metabolites. β -oxidation of the side chain did not appear to be a metabolic pathway in rotational crops.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop data are classified as scientifically acceptable. The acceptability of these studies for regulatory purposes will be addressed in the forthcoming U.S. EPA 2,4-DB Reregistration Eligibility Decision Document, Residue Chemistry Chapter.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

2,4-DB is a FIFRA List A pesticide active ingredient classified as a plant growth regulator and



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herbicide. The 2,4-DB nomenclature is listed in Table A.1. The physicochemical properties of the technical grade of 2,4-DB are presented in Table A.2.

TABLE A.1. 2,4-DB Nomenclature	
Compound	Chemical Structure
Common name	2,4-DB
Company code number	None specified
IUPAC name	4-(2,4-dichlorophenoxy)butyric acid
Chemical abstract name	4-(2,4-dichlorophenoxy)butanoic acid
CAS registry number	94-82-6
End-use products/EP	Refer to the Residue Chemistry Chapter of the 2,4-DB RED

TABLE A.2. Physicochemical Properties of the Technical Grade of 2,4-DB		
Parameter	Value	Reference
Melting point/range	113.5-117.5 °C	D198970, 9/30/94, F. Toghrol
pH (20 °C)	4.31 (1% w:v)	D198970, 9/30/94, F. Toghrol
Density (22 °C)	1.461	D198970, 9/30/94, F. Toghrol
Water solubility (25 °C)	46 ppm	D161375, 7/9/91, F. Toghrol
Solvent solubility (25 °C)	100 g/L in acetone 50 g/L in ethanol	D161375, 7/9/91, F. Toghrol
Vapor pressure	7.1×10^{-7} mm Hg at 23.6 °C	DEB No. 6823, 8/3/90, G. Makhijani
Dissociation constant, pK_a	2.99×10^{-5}	DEB No. 6823, 8/3/90, G. Makhijani
Octanol/water partition coefficient, $\text{Log}(K_{ow})$	2.62	DEB No. 6823, 8/3/90, G. Makhijani
UV/visible absorption spectrum	Three distinct bands at 202, 229, and 284 nm; no appreciable differences in the extinction coefficients between acidic, neutral, and basic solutions.	D286840, 1/9/03, S. Malak

B. EXPERIMENTAL DESIGN

Information pertaining to the testing environment and location as well as soil characteristics is presented in Table B.1.1. Information pertaining to rotational crop varieties and harvesting procedures is detailed in Table B.1.2. Information pertaining to the radiolabeled test material is listed in Table B.2.1. The use pattern information is presented in Table B.3.1.



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B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information							
Testing Environment and location	Soil characteristics (0-30 inches depth)						
	Type	% Sand	% Silt	% Clay	% OM	pH	CEC (Meg/100 g)
Two large fenced outdoor plots, each containing 3 subplots (2x20 ft) at Pan-Agricultural Research Station (Madera, CA).	Hanford sandy loam	63.2-71.3	19.2-25.2	7.5-11.5	0-0.5	6.2-6.7	5.6-6.4

Daily minimum and maximum temperatures and precipitation were reported for the duration of the study, and plots were irrigated as needed using overhead sprinklers from an on-site well. Historical 30-year (1960-1989) monthly average temperatures were included. No unusual weather conditions were reported.

TABLE B.1.2. Crop Information					
Crop/ crop group	Variety	Plantback intervals (days)	Growth stage at harvest	Harvested RAC	Harvesting procedure
Barley/Cereal grains and forage, fodder, and straw of cereal grains	<i>Hordeum vulgare</i> , var. NK-BB82-2	30, 120, and 365	Immature (25-111 DAP ¹); mature (160-230 DAP)	Immature forage, and mature straw and grain	None
Carrot/Root and tuber, and leaves of root and tuber vegetables	<i>Daucus carota</i> , var. Emperor 58		Immature (90-194 DAP); mature (160-228 DAP)	Tops and roots	Whole plants collected.
Lettuce/Leafy (except Brassica) vegetables	<i>Lactuca sativa</i> , var. Waldmaure Greenleaf		Immature (38-119 DAP); mature (66-140 DAP)	Leaves	Cut leaves above the soil surface.

¹ DAP = Days after planting.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics	
Chemical structure	
Radiolabel position	Uniformly in the phenyl ring
Batch No.	Lot CSL 2567150



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TABLE B.2.1. Test Material Characteristics

Radiochemical purity	98.8%
Specific radioactivity	13.03 mCi/mmol; 52.33 μ Ci/mg (22,644 dpm/ μ g) test substance
Code No. of specific batch	PTRL No. 508-1

B.3. Study Use Pattern**TABLE B.3.1. Use Pattern Information**

Chemical name	[phenyl-U- ¹⁴ C]2,4-DB
Composition of spray mixture	The radiolabeled material was dissolved in acetone and isotopically diluted with nonlabeled 2,4-DB.
Application method	The test substance was applied as a broadcast spray to the soil surface using a CO ₂ backpack sprayer, followed by a water rinse spray of equal volume. The control plot was sprayed with a solution of formulation blank, Triton X-100, water, and acetone.
Application rate	The target application rate was 1.5 lb ai/A. The verified application rate was 1.65 lb ai/A.
Number of applications	One
Timing of applications	30 days prior to the first planting rotation
PHI	N/A; application to bare soil

B.4. Identification/Characterization of Residues**B.4.1. Crops Sample Preparation**

Immature and mature carrot samples were separated into tops and roots; if necessary the roots were dipped in water to remove excess surface soil and were blotted dry. Mature barley samples were separated into grain and straw. All samples from the 30-day rotation were extracted for metabolite characterization, but only the 120-day PBI carrot top (immature and mature), carrot root (immature), and barley forage, straw, and grain, and 365-day PBI barley and straw rotated crop samples were extracted.

Plant samples were first chopped with dry ice and frozen. Residues were repeatedly (3x) extracted with methanol:water (1:1, v:v) using a Brinkman Polytron and then filtered. The remaining filter cake was further extracted (2x) with saturated ethyl acetate (EtOAc) and filtered. The solids (nonextractable residues) remaining after solvent extractions were then subjected to sequential acid (0.1 N HCl at reflux for 4 hours) and base (0.1 N NaOH at reflux for 4 hours) hydrolysis and filtered. The acid and base hydrolysates were separately partitioned (3x) with saturated EtOAc which yielded organo- and aqueous-soluble fractions. In general, extracts and hydrolysates containing >0.01 ppm radioactivity were concentrated for HPLC analysis.

B.4.2. Analytical Methodology

Samples of rotational crop commodities were subjected to combustion/LSC (liquid scintillation



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counter) for TRR determination. Extracts and hydrolysates of rotated crop samples were radioassayed directly by LSC, and nonextractable radioactivity was determined by combustion/LSC.

The methanol:water extracts of immature lettuce, immature carrot tops, and barley forage samples from the 30-day PBI as well as the barley straw samples from the 120-day PBI were analyzed by HPLC; the radioactivity in all of the 365-day PBI sample extracts was low and did not warrant further analysis. Only the base hydrolysate of the 120-day barley forage contained sufficient radioactivity for HPLC analysis. The reversed phase HPLC systems used were equipped with a Supelco (RP C18) column and UV detector. Gradient mobile phases of methanol:acetonitrile (1:1, v:v) and 0.005 M KH_2PO_4 (pH 3) were used. HPLC fractions were collected, and radioactivity was determined by subsequent LSC or by direct radioassay using a radioactivity flow monitor. The following reference standards were used:

Radiolabeled standards: 2,4-dichlorophenoxybutyric acid; 2,4-dichlorophenoxyacetic acid; and 2,4-dichlorophenol.

Nonradiolabeled standards: 2,4-DB acid; 2,4-D; 2,4-D phenol; 4-chlorophenol; 2-chloro-4-hydroxyphenol; 4-chlorophenoxyacetic acid; 2,3-dichloroanisole; 4,6-dichlororesorinol; 1,4-benzoquinone; and 2,5-dichloro-4-hydroxyphenol.

Synthesized nonradiolabeled standards: 2,4-dichloro-6-hydroxyphenol; 2,3-dichloro-4-hydroxyphenol; 2,4-dichloro-3-hydroxyphenol; 2,4-dichloro-5-hydroxyphenoxyacetic acid; 2,5-dichloro-4-hydroxyphenoxyacetic acid; 2,4-dichloro-6-hydroxyphenoxyacetic acid; 2,3-dichloro-4-hydroxyphenoxyacetic acid; 2,4-dichloro-3-hydroxyphenoxyacetic acid; 2,3-dichloro-4-hydroxyphenoxybutyric acid; 2,4-dichloro-5-hydroxyphenoxybutyric acid; 3,5-dichloro-5-hydroxyphenoxybutyric acid; 2,4-dichloro-6-hydroxyphenoxybutyric acid; 2,5-dichloro-4-hydroxyphenoxybutyric acid; 6-(2,4-dichlorophenoxy)hexanoic acid; 4-(2,4-dichlorophenoxy)methyl crotonate; and 10-(2,4-dichlorophenoxy)decanoic acid.

The identification of certain metabolites was confirmed by TLC analysis. The normal phase TLC system utilized silica gel 60 plates and a solvent phase of butanol:acetic acid:water (10:1:1, v:v:v). UV light was used to visualize co-spotted reference standards, and radioactivity was detected using an Imaging Scanner.

To determine the nature of bound residues, the nonextractable residues in 365-DAT barley forage was subjected to cell wall fractionation into various components. The solids (nonextractable residues) were sequentially subjected to buffer extraction, starch digestion, protein digestion, pectin extraction, lignin extraction, hemicellulose extraction, and cellulose hydrolysis. A brief discussion of each extraction/digestion follows; following each step, the extract was filtered and the residue on the filter wash was carried onto the next extraction/digestion step.

- Buffer extraction with potassium phosphate buffer (pH 7), shaken for 10 minutes.
- Starch digestion with α -amylase, incubated at 30 °C for ~20 hours.



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- Protein digestion with Pronase E, incubated at 25 °C for ~20 hours.
- Pectin extraction with sodium acetate/EDTA (pH 4.5), incubated at 80 °C for ~6 hours.
- Lignin extraction with glacial acetic acid and sodium chlorite, incubated at 70 °C for ~1 hour; repeated three more times and the filtrate adjusted to pH 7-9 with 1 M NaOH.
- Hemicellulose extraction with 24% potassium hydroxide, shaken for 24 hours; the pH was adjusted to 4.5 with 6 N acetic acid, shaken for 1 hour, and centrifuged.
- Cellulose hydrolysis with sulfuric acid, shaken for ~4 hours; 24% potassium hydroxide was added and vortexed; the pH was adjusted to 6.5-7.5 with additional potassium hydroxide, and water was added until the precipitated potassium sulfate dissolved.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in rotational crops are reported in Table C.2.1. At the 30-day PBI, TRR ranged from 0.01 to 0.06 ppm. At the 120-day PBI, TRR ranged from 0.01 to 0.07 ppm. At the 365-day PBI, TRR ranged from <0.01 to 0.02 ppm. There was no clear pattern of decline in TRR levels at longer PBIs.

The distribution of radioactivity in rotated crop matrices, which were subjected to residue extraction, is presented in Tables C.2.2.1 (30-day PBI), C.2.2.2 (120-day PBI), and C.2.2.3 (365-day PBI). Solvent extraction released about 20-82% of TRR from crop matrices. Additional radioactivity (6-22% TRR) was released with acid and base hydrolysis; nonextractable residues were 9-101% TRR (<0.01-0.02 ppm). Material balances ranged 67-169% in the rotated crop matrices.

The characterization and identification of residues are summarized in Table C.2.3. Only the extracts of the 30-day PBI immature lettuce, immature carrot top, and barley forage samples and 120-day PBI barley straw samples were analyzed by HPLC for residue identification. The total identified residues comprised <7% of TRR in the analyzed matrices. The parent, 2,4-DB was not detected in any rotated crop matrices. Identified residues were each present at <0.01 ppm; 1,4-BQ was identified in immature lettuce (1.63% TRR) and barley straw (2.30% TRR); CHQ was identified in barley forage (2.34% TRR); and 2,3-Cl-4-HPAA was identified in barley forage (1.20% TRR) and straw (0.83% TRR). Metabolites (2,5-dichloro-4-hydroxy and 2,4-dichloro-3-hydroxy)phenoxy acetic acid and 2,5-dichloro-4-hydroxyphenol could not be separated for identification and were detected in barley straw at 2.54% TRR (<0.01 ppm). Metabolites (2,4-dichloro-3-hydroxy and 2,3-dichloro-4-hydroxy)phenol also could not be separated for identification and were detected at <0.01 ppm in barley forage (0.84% TRR) and straw (1.19% TRR). In addition, up to 3 unknowns were characterized in immature lettuce, immature carrot tops, barley forage, and barley straw; each unknown was present at ≤0.01 ppm.

Only the base hydrolysate of 120-day barley forage was analyzed by HPLC for metabolite identification. 2,4-Dichlorophenol was identified at 10.91% TRR (<0.01 ppm), and two unknowns accounting for 4.10% TRR (<0.01 ppm) were characterized.



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Only the 365-day barley forage nonextractable residues were further characterized by cell wall fractionation. The nonextractable residues were characterized as primarily cellulose (~26% TRR) and hemicellulose (~7% TRR). Starch and pectin (each <1% TRR), protein (~2% TRR), and lignin (<2% TRR) were also observed at minor levels in the bound residues of barley forage.

The study submission provided data pertaining to characterization and identification of residues in soil which are not included in this Data Evaluation Record.

C.1. Storage Stability

Rotational crop samples were stored frozen prior to TRR determinations and extraction. All extracts were stored refrigerated until analysis; extraction and analysis dates were not provided. To demonstrate the stability of [^{14}C]2,4-DB in plant matrices, a sample of untreated immature barley forage (30-day PBI) was fortified at the field site with [^{14}C]2,4-DB and stored frozen for the duration of the study (1,089 days). The stored sample was extracted through the methanol:water steps and analyzed by HPLC. Analysis at the study conclusion, quantitated 83% 2,4-DB, 2% 2,4-dichlorophenol, 3% 2,4-dichlorophenoxy acetic acid, and 2% 4-chlorophenol. Approximately 10% of the radioactivity eluted early as a polar component. These data demonstrate that [^{14}C]2,4-DB is relatively stable in barley forage for up to ~36 months, and support the storage conditions and intervals of samples from the rotational crop study.

TABLE C.1. Summary of Storage Conditions

Matrix [RAC or Extract]	Plantback interval (days)	Storage Temp.	Actual Storage Duration	Limit of Demonstrated Storage Stability
Barley forage, straw, and grain; carrot tops and roots; lettuce	30, 120, 365	<-5 °C	Extraction and analysis dates were not provided; however the registrant stated that the study duration was ~36 months	1089 days (35.8 months) for [^{14}C]2,4-DB in barley forage.



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C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Rotational Crop Matrices.			
Matrix	Plantback interval (days)	TRR (ppm)	
		Immature Samples	Mature Samples
Carrot, tops	30	0.02	0.01
	120	0.03	0.02
	365	0.01	0.01
Carrot, roots	30	0.02	0.01
	120	0.01	0.01
	365	<0.01	<0.01
Lettuce	30	0.05	0.03
	120	0.02	0.02
	365	0.01	0.01
Barley, forage	30	0.06	--
	120	0.03	--
	365	0.02	--
Barley, straw	30	--	0.01
	120	--	0.07
	365	--	0.02
Barley, grain	30	--	0.01
	120	--	0.03
	365	--	0.01



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TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 30 Days Following Application of [Phenyl-¹⁴C]2,4-DB to the Soil. ¹

Metabolite Fraction	Immature Lettuce (30-day PBI)		Mature Lettuce (30-day PBI)		Immature Carrot Tops (30-day PBI)		Immature Carrot Root (30-day PBI)		Mature Carrot Tops (30-day PBI)		Mature Carrot Root (30-day PBI)	
	(TRR = 0.05 ppm)		(TRR = 0.03 ppm)		(TRR = 0.02 ppm)		(TRR = 0.02 ppm)		(TRR = 0.01 ppm)		(TRR = 0.01 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol:water	16	0.01	46	0.01	22	<0.01	43	0.01	68	0.01	59	0.01
1,4-BQ	1.63	<0.01			--	--						
Unknown 1 (Rt 5-6 mins)	12.51	0.01			--	--						
Unknown 2 (Rt 6.5-7 mins)	--	--			22.00	<0.01						
Unknown 3 (Rt 7.5-8 mins)	1.87	<0.01			--	--						
Ethyl acetate	6	<0.01	11	<0.01	10	<0.01	17	<0.01	14	<0.01	8	<0.01
Acid (0.1 N HCl) hydrolysate	4	<0.01	14	<0.01	5	<0.01	12	<0.01	5	<0.01	14	<0.01
-Aqueous phase							7	<0.01				
-Organic phase							3	<0.01				
Base (0.1 N NaOH) hydrolysate	4	<0.01	5	<0.01	3	<0.01	6	<0.01	6	<0.01	8	<0.01
-Aqueous phase							5	<0.01				
-Organic phase							6	<0.01				
Total extractable	30	<0.02	76	<0.02	40	<0.01	78	<0.02	93	0.01	89	0.01
Total identified	1.63	<0.01	0	0	0	0	0	0	0	0	0	0
Total unidentified	28.38	<0.02	76	<0.02	40	<0.01	78	<0.02	93	0.01	89	0.01
Total bound residues (PES)	37	0.02	9	<0.01	50	0.01	32	<0.01	52	<0.01	32	<0.01
% Accountability	67		85		90		110		145		121	



TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 30 Days Following Application of [Phenyl- ¹⁴ C]2,4-DB to the Soil (continued). ¹						
Metabolite Fraction	Barley Forage (30-day PBI)		Barley Straw (30-day PBI)		Barley Grain (30-day PBI)	
	(TRR = 0.06 ppm)		(TRR = 0.01 ppm)		(TRR = 0.01 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol:water	20	0.01	19	<0.01	20	<0.01
CHQ	2.34	<0.01			--	--
2,3-Cl-4-HPAA	1.20	<0.01			--	--
(2,4-dichloro-3-hydroxy or 2,3-dichloro-4-hydroxy)phenol	0.84	<0.01			--	--
Unknown 1 (Rt 5-6 mins)	13.24	0.01			39.00	0.01
Unknown 3 (Rt 7.5-8 mins)	2.36	<0.01			--	--
Ethyl acetate	6	<0.01	6	<0.01	53	0.01
Acid (0.1 N HCl) hydrolysate	8	<0.01	10	<0.01	4	<0.01
-Aqueous phase						
-Organic phase						
Base (0.1 N NaOH) hydrolysate	2	<0.01	7	<0.01	2	<0.01
-Aqueous phase						
-Organic phase						
Total extractable	36	<0.02	42	<0.01	79	0.01
Total identified	4.38	<0.01	0	0	0	0
Total unidentified	31.60	<0.02	42	<0.01	79	0.01
Total bound residues (PES)	32	0.02	45	<0.01	70	<0.01
% Accountability	68		87		149	

¹ Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.



TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 120 Days Following Application of [Phenyl-¹⁴C]2,4-DB to the Soil. ¹

Metabolite Fraction	Immature Carrot Tops (120-day PBI)		Immature Carrot Root (120-day PBI)		Mature Carrot Tops (120-day PBI)		Barley Forage (120-day PBI)		Barley Straw (120-day PBI)		Barley Grain (120-day PBI)	
	(TRR = 0.03 ppm)		(TRR = 0.01 ppm)		(TRR = 0.02 ppm)		(TRR = 0.03 ppm)		(TRR = 0.07 ppm)		(TRR = 0.03 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol:water	26	0.01	38	<0.01	31	0.01	24	0.01	22	0.01	16	0.01
1,4-BQ									2.30	<0.01		
2,3-Cl-4-HPAA									0.83	<0.01		
(2,5-dichloro-4-hydroxy or 2,4-dichloro-3-hydroxy) phenoxy acetic acid or 2,5-dichloro-4-hydroxyphenol									2.54	<0.01		
(2,4-dichloro-3-hydroxy or 2,3-dichloro-4-hydroxy)phenol									1.19	<0.01		
Unknown 1 (Rt 5-6 mins)									3.64	<0.01		
Unknown 2 (Rt 6.5-7 mins)									2.81	<0.01		
Unknown 3 (Rt 7.5-8 mins)									4.68	<0.01		
Ethyl acetate	6	<0.01	9	<0.01	8	<0.01	12	<0.01	6	<0.01	4	<0.01
Acid (0.1 N HCl) hydrolysate	6	<0.01	14	<0.01	5	<0.01	7	<0.01	3	<0.01	1	<0.01
-Aqueous phase							1	<0.01				
-Organic phase							1	<0.01				
Base (0.1 N NaOH) hydrolysate	5	<0.01	7	<0.01	7	<0.01	15	0.01	3	<0.01	9	<0.01
2,4-dichlorophenol							10.91	<0.01				
Unknowns							4.10	<0.01				
-Aqueous phase							8	<0.01				
-Organic phase							3	<0.01				
Total extractable	43	<0.02	68	<0.01	51	<0.02	58	<0.02	34	<0.02	30	<0.02
Total identified	0	0	0	0	0	0	10.91	<0.01	6.86	<0.01	0	0
Total unidentified	43	<0.02	68	<0.01	51	<0.02	47.10	<0.02	23.13	<0.01	30	<0.02
Total bound residues (PES)	66	0.02	101	0.01	63	0.01	38	0.01	53	0.03	38	0.01



TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 120 Days Following Application of [Phenyl- ¹⁴ C]2,4-DB to the Soil. ¹												
Metabolite Fraction	Immature Carrot Tops (120-day PBI)		Immature Carrot Root (120-day PBI)		Mature Carrot Tops (120-day PBI)		Barley Forage (120-day PBI)		Barley Straw (120-day PBI)		Barley Grain (120-day PBI)	
	(TRR = 0.03 ppm)		(TRR = 0.01 ppm)		(TRR = 0.02 ppm)		(TRR = 0.03 ppm)		(TRR = 0.07 ppm)		(TRR = 0.03 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
% Accountability	109		169		114		96		87		68	

¹ Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.



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TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 365 Days Following Application of [Phenyl- ¹⁴ C]2,4-DB to the Soil. ¹				
Metabolite Fraction	Barley Forage (365-day PBI)		Barley Straw (365-day PBI)	
	(TRR = 0.02 ppm)		(TRR = 0.02 ppm)	
	%TRR	ppm	%TRR	ppm
Methanol:water	34	0.01	17	<0.01
Ethyl acetate	20	<0.01	36	0.01
Acid (0.1 N HCl) hydrolysate	5	<0.01	3	<0.01
-Aqueous phase	2	<0.01		
-Organic phase	5	<0.01		
Base (0.1 N NaOH) hydrolysate	2	<0.01	3	<0.01
-Aqueous phase	3	<0.01		
-Organic phase	4	<0.01		
Nonextractable ²	37	0.01	58	0.01
-Initial wash with potassium phosphate pH 7 buffer	<0.37	NR ³		
-Starch	0.74	NR		
-Protein	2.22	NR		
-Pectin	0.37	NR		
-Lignin	1.48	NR		
-Hemicellulose	6.66	NR		
-Cellulose	25.9	NR		
Total extractable	61	<0.02	59	<0.02
Total identified	0	0	0	0
Total unidentified	61	<0.02	59	<0.02
Total bound residues (PES)	NR	NR	58	0.01
% Accountability	98		117	

¹ Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.

² Nonextractable residues were characterized by cell wall fractionation; % of total bound radioactivity was reported by the registrant and recalculated as % TRR by the study



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reviewer.

³ NR = Not reported.

**TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Following Application of Radiolabeled 2,4-DB to the Soil at 1.65 lb ai/A. ¹**

Compound	Immature Lettuce (30-day PBI)		Immature Carrot Tops (30-day PBI)		Barley Forage (30-day PBI)		Barley Forage (120-day PBI)		Barley Straw (120-day PBI)		Barley Forage (365-day PBI)	
	(TRR = 0.05 ppm)		(TRR = 0.02 ppm)		(TRR = 0.06 ppm)		(TRR = 0.03 ppm)		(TRR = 0.07 ppm)		(TRR = 0.02 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
1,4-BQ	1.63	<0.01	--	--	--	--	--	--	2.30	<0.01		
CHQ	--	--	--	--	2.34	<0.01	--	--	--	--		
2,3-CI-4-HPAA	--	--	--	--	1.20	<0.01	--	--	0.83	<0.01		
2,4-dichlorophenol	--	--	--	--	--	--	10.91	<0.01	--	--		
(2,5-dichloro-4-hydroxy or 2,4-dichloro-3-hydroxy)phenoxy acetic acid or 2,5-dichloro-4-hydroxyphenol	--	--	--	--	--	--	--	--	2.54	<0.01		
(2,4-dichloro-3-hydroxy or 2,3-dichloro-4-hydroxy)phenol	--	--	--	--	0.84	<0.01	--	--	1.19	<0.01		
Unknowns	14.38	<0.02	22.00	<0.01	15.6	<0.01	4.10	<0.01	11.13	<0.01		
Methanol:water	--	--	--	--	--	--	24	0.01	--	--	34	0.01
Ethyl acetate	6	<0.01	10	<0.01	6	<0.01	12	<0.01	6	<0.01	20	<0.01
Acid hydrolysate	4	<0.01	5	<0.01	8	<0.01	7	<0.01	3	<0.01	5	<0.01
Base hydrolysate	4	<0.01	3	<0.01	2	<0.01	--	--	3	<0.01	2	<0.01
Potassium phosphate wash											<0.37	NR
Starch											0.74	NR
Protein											2.22	NR
Pectin											0.37	NR
Lignin											1.48	NR
Hemicellulose											6.66	NR
Cellulose											25.9	NR
Total identified	1.63	<0.01	0	0	4.38	<0.01	10.91	<0.01	6.86	<0.01	0	0
Total characterized	28.38	<0.02	40	<0.01	31.60	<0.02	47.10	<0.02	23.13	<0.01	61	<0.02
Total extractable	30	<0.02	40	<0.01	36	<0.02	58	<0.02	34	<0.02	61	<0.02
Total bound	37	0.02	50	0.01	32	0.02	38	0.01	53	0.03	NR	NR



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¹ Shading indicates that the identification and/or characterization analysis was not conducted for the matrix in question. NR = Not reported.



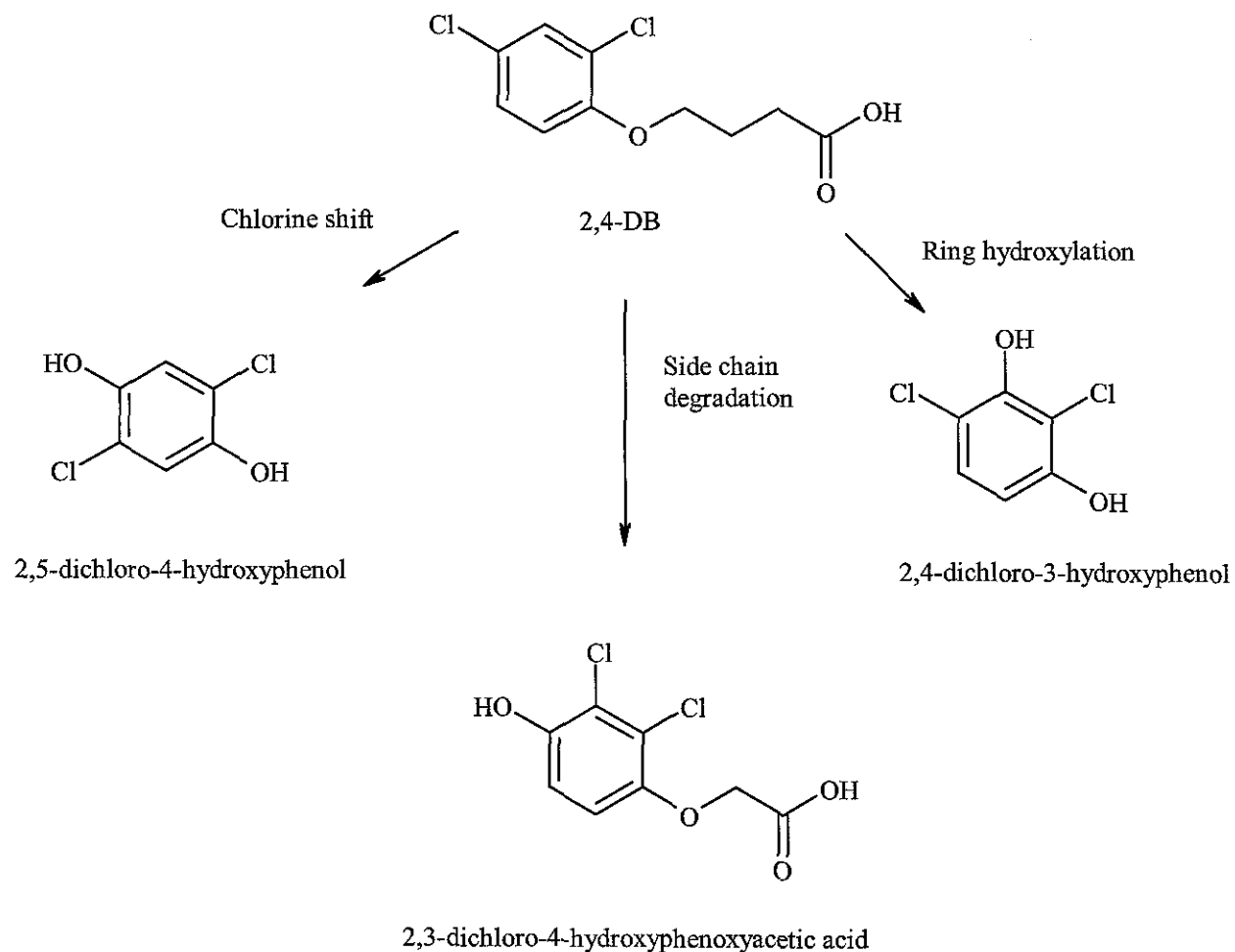
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C.3. Chemical Structures of Metabolites Identified from the Confined Rotational Crop Studies

FIGURE C.3.1. Proposed Metabolic Profile of 2,4-DB in Rotational Crops.



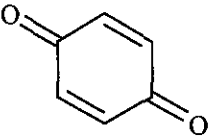
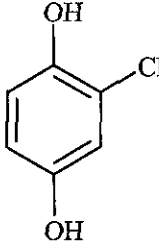
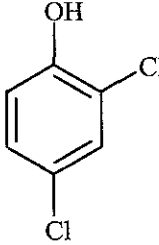
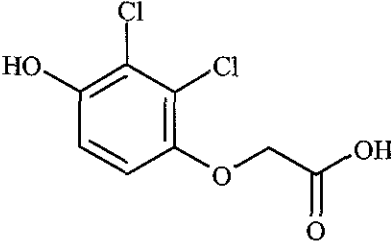
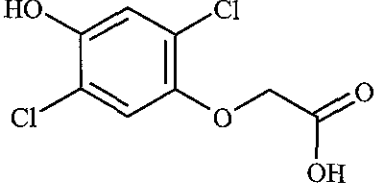
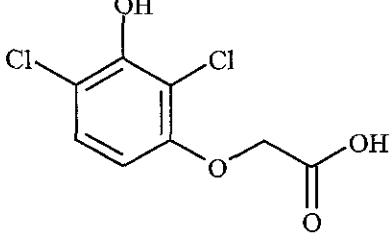


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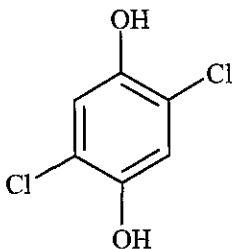
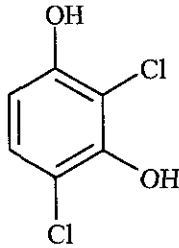
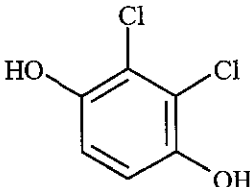
TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study

Common name/code	Chemical name	Chemical structure
1,4-BQ	1,4-benzoquinone	
CHQ	2-chloro-4-hydroxyphenol	
2,4-D phenol	2,4-dichlorophenol	
2,3-Cl-4-HPAA	2,3-dichloro-4-hydroxyphenoxyacetic acid	
2,5-Cl-4-HPAA	2,5-dichloro-4-hydroxyphenoxyacetic acid	
2,4-Cl-3-HPAA	2,4-dichloro-3-hydroxyphenoxyacetic acid	



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TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study		
Common name/code	Chemical name	Chemical structure
2,5-Cl-4-HOH	2,5-dichloro-4-hydroxyphenol	
2,4-Cl-3-HOH	2,4-dichloro-3-hydroxyphenol	
2,3-Cl-4-HOH	2,3-dichloro-4-hydroxyphenol	

D. CONCLUSION

Uniformly ring labeled [^{14}C]2,4-DB was applied to bare sandy loam soil at 1.65 lb ai/A. Following 30, 120, and 365 days of aging under outdoor conditions, barley, carrots, lettuce were sown onto the treated soil. TRR accumulated at ≥ 0.01 ppm in various rotated crops planted at the 30, 120, and 365-day plantback intervals, but were very low (maximum of 0.07 ppm in 120-day barley straw).

Select samples from the 30-, 120-, and 365-day rotations were extracted with organic solvents and subjected to acid and base hydrolysis. Analysis of the extracts by HPLC identified the metabolites 1,4-BQ, CHQ, 2,3-Cl-4-HPAA, a mixture of (2,5-dichloro-4-hydroxy and 2,4-dichloro-3-hydroxy)phenoxy acetic acid with 2,5-dichloro-4-hydroxyphenol, and a mixture of (2,4-dichloro-3-hydroxy and 2,3-dichloro-4-hydroxy)phenol. 2,4-Dichlorophenol was also identified in the base hydrolysate of barley forage. The parent, 2,4-DB, was not identified in any rotated crop matrices. The nonextractable residues in barley forage were characterized as primarily cellulose and hemicellulose, with minor levels attributed to starch, protein, pectin, and lignin.

The confined rotational crop studies showed that no significant residues of 2,4-DB are taken up in succeeding rotational crops. The registrant proposes that the major metabolic pathways in



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rotational crop matrices encompass side chain degradation, chlorine shift and ring hydroxylation as demonstrated by the observed metabolites. β -oxidation of the side chain did not appear to be a metabolic pathway in rotational crops.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: J.Morales (10/21/03); W. Phang (11/13/03);

Petition Number(s): None

DP Barcode(s): D290502

PC Code: 030801

Template Version April 2003



13544

R096567

Chemical: 4-(2,4-Dichlorophenoxy)butyric acid

PC Code: 030801

HED File Code 11000 Chemistry Reviews

Memo Date: 11/13/2003

File ID: DPD290502

Accession Number: 412-04-0139

HED Records Reference Center
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